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IMPORTANCE OF BIOLOGICAL SYSTEMS IN INDUSTRIAL WASTE TREATMENT

POTENTIAL APPLICATION TO THE SPACE STATION

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INTRODUCTION

Hazardous chemicals in the environment are a global concern and in recent years this subject has received considerable attention. The Congressional Office of Technology Assessment (OTA) (Hirschhorn et al. 1983) estimates that the costs for cleaning up existing sites containing hazardous chemicals could range from 80 to 300 billion dollars using conventional technology. These projected costs have stimulated several federal agencies to support research into the development of less expensive methods for treating hazardous chemicals. In recent years, with the support of the Federal Government, several such technologies have been developed, including incineration and vitrification. Although these technologies are effective in treating hazardous waste they are expensive.

An additional technology which is currently being developed is the use of microbial systems for the degradation and/or immobilization of hazardous waste. This latter technology has been shown to be effective in the treatment of some hazardous chemicals at costs that are less than conventional technologies.

Microbial degradation of waste is perhaps one of the oldest waste management system known to man. This system has been used for

centuries to treat human waste (e.g., sewage). In fact, the majority of organic waste products, both natural and synthetic, are probable degraded by microorganisms (Alexander, 1981). Since many hazardous organics are related chemically to naturally occurring chemicals it is not surprising that microorganisms capable of degrading hazardous organic chemicals have been isolated from the environment (Cook et al., 1983; Ahmed and Focht, 1973; Fedorak and Westlake, 1984; Johnson and Talbot, 1983). Microorganisms capable of immobilizing hazardous chemicals through biological processes which facilitate adsorption, absorption and/or conversion of the chemicals have also been identified in the environment (Revis et al., 1988; Postgate, 1984).

Although several microorganisms isolated from the environment have been shown to degrade and/or immobilize hazardous chemicals the rates at which some chemicals are degraded and immobilized is very slow. For example, Brown et al. (1988) estimates that it would take approximately 300 years for microbial systems in the sediment from the Hudson River to completely dechlorinate existing concentrations of polychlorinated biphenyls (PCBs). Such slow rates would reduce the cost advantages of industrial application of microbial systems.

In an attempt to increase the rates of microbial degradation of organic chemicals, several investigators are exploring biochemical pathways in microorganisms capable of degrading the organics (albeit at slow rates) with the hope of amplifying these pathways through genetics engineering. Since many of the degrading pathways require enzymes to metabolize the organics scientists are investigating methods for

increasing the rate of synthesis of the degrading enzymes. In a recent study Rojo et al. (1988) engineered a Pseudomonas species capable of degrading chloro- and methylaromatics. This organism, prior to genetic engineering, was only capable of degrading chloroaromatics at relatively slow rates. However, after engineering, this strain the bacteria was capable of degrading both the chloro and methylaromatics at significantly increased rates. Thus, genetic engineering may provide a method for increasing the rate at which microorganisms degrade hazardous organics. Because microbial systems used in waste treatment are generally less expensive than the conventional chemical and physical methods, further development of engineered organisms may prove to be the method of choice for treating hazardous chemicals in the environment.

In addition to having applications for waste management issues on planet earth, microbial systems may have application in reducing waste volumes aboard space craft. A candidate for such an application is the space station. The National Aeronautics and Space Administration plans to launch a space station by the year 2000. The station will serve to support the space research efforts of the United States and several western countries. Many of the planned experiments will generate aqueous waste. Because of space and weight limitations in the space station a need exists for: 1) minimizing the amount of aqueous waste generated, 2) recycling of air and 3) recycling water. To recycle air and water the contaminants from previous experiments must be removed before the air and water can be used for other experiments. This goal may be achieved using microorganisms in a bioreactor.

Contaminants in the air and water may be removed via degradation, adsorption, absorption and/or precipitation by microorganisms. The microorganisms and contaminants are then both removed from the aqueous phase using ultra filtration. The air and water are recycled and the sludge is solidified by heating. It is of interest to note that water vapor from the heated sludge can also be collected if the contaminants in the sludge do not volatilize.

Potential Bioreactors for the Space Station

a) Inorganics

Described in Table 1 is a list of potential contaminants that may be found in air and aqueous waste generated from experiments planned for the space station. For the inorganics, several microbial systems have been shown to be effective in precipitating metals from solution. These metals include mercury, silver, cadmium, nickel copper, iron, and aluminum. However systems have not been identified, to the author's knowledge that precipitate gallium, tellurium, germanium, niobium, and indium. For arsenic, beryllium, silicon, and tungsten, microbial systems have been identified which adsorb and/or absorb these metals.

b) Organics

Several microbial systems have shown to degrade the organics methanol, ethanol, acetone, benzene, trichloroethylene, xylene, and toluene (Table 1). Systems can also absorb and/or adsorb the organics

dimethyl sulfoxide, acetonitrile, trichloroethylene, carbon tetrachloride and sodium azide. To the authors knowledge microbial systems which degrade the organics glutaraldehyde, chlorodifluoromethane, trichlorotrifluoroethane, and methyl ethyl ketone remain to be reported. However based on reports in the literature which describe microbes which can degrade chloroorganics, it is likely that microbes will eventually be found that can degrade chloro and fluoro compounds.

c) Etchants

A sulfate-reducing bacterium that metabolizes nitric and sulfuric acid to nitrogen and sulfide, respectively, would be an appropriate microbial system for reducing the levels of these acids in the aqueous waste. Furthermore, sulfuric acid could be used as a sulfate source in a bioreactor designed for the precipitation of metals through the formation of metal sulfides.

It is thus possible to reduce the concentration of many of the contaminants shown in Table 1 using microorganisms in a bioreactor. The microbial systems identified above for the organics, inorganics and etchants include both aerobes and anaerobes. Thus it may be necessary to maintain two bioreactors (e.g. an aerobic and anaerobic reactor).

ANTICIPATED WASTE FROM EXPERIMENTS PROPOSED FOR THE SPACE STATION

ORGANICS	INORGANICS	ETCHANTS
Acetonitrile,	Gallium, Iron	Acids
Ethanol, Methanol,	Arsenic,	Nitric
Acetone, Benzene,	Cadmium,	Hydrofluoric
Toluene, Xylene,	Germanium,	Sulfuric
Dimethyl sulfoxide	Tantalum,	
Dimethyl formamide	Beryllium,	Bases
Trichloroethylene,	Copper,	Sodium
Carbon tetra-	Aluminum,	Potassium
chloride, Na azide	Silver,	
Glutaraldehyde,	Indium,	Hydrogen
Chloroacetylene-	Tungsten,	Bromide
methane, Trichloro-	Mercury,	Chloride
trifluoroethane, &	Tellurium, &	
Methyl ethylketone	Niobium	

RESULTS AND CONCLUSIONS

Sulfate-reducing bacteria reduce sulfate to sulfide under anaerobic conditions and in this process they derive energy. The sulfide is released from the bacteria as hydrogen sulfide (Figure 1). Following the displacement of hydrogen the sulfide will react with a variety of metals to give a metal sulfide. Since metal sulfides are relatively insoluble (Table 2) in aqueous solution they precipitate and the metals can be recovered through ultra filtration of the solution.

These bacteria will grow in solution containing metals at concentrations ranging from 0 to 10,000 ppm. An example of the effect of sulfate-reducing bacteria on metals is shown in Table 3. In this experiment bacteria were added to a solution containing sulfate, lead, cadmium, mercury, nickel, copper, and zinc. After incubation at 32 °C for 14 h., the mixture was filtered and analyzed for the above metals. As shown, the filtration process reduced the concentration of all of the added metals. That these metals were not filterable suggests that they are in the sulfide form. Thus, metals which form metal sulfides are potential candidates for treatment with the sulfate-reducing bacteria. Since the metals gallium, tellurium, germanium, tantalum and indium can exist in the sulfide form they would be candidates for sulfide precipitation by sulfate-reducing bacteria. However experiments would be necessary to confirm this suggestion.

Sulfate-Reducing Bacteria Effect: **Toxic metals in soil, sediment, sludge**

SULFATE-REDUCING BACTERIA

+ sulfate and nutrients

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HYDROGEN SULFIDE

+

IONIC METALS

in soil, sediment or sludge

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METAL SULFIDE

Metals, Cd, Hg, Pb, Cu, Ag, Ni, Sn

Solubility of Metal Sulfides

in aqueous solution

Metal	Metal Sulfide	Solubility ug/liter
Cadmium	CdS	.00000087
Cobalt	CoS	.000016
Copper	CuS	.000000000000088
Lead	PbS	.0000043
Mercury	HgS	.0000000000000011
Nickel	NiS	.00011
Silver	Ag ₂ S	.0000000084
Tin	SnS	.000043

Precipitation Heavy Metals by Sulfate Reducing Bacteria

Metal	Permit Level ppb	Concentration after filtration ppb	
		Starting	Final
Cadmium	1000 *	500	2.5
Mercury	200 *	100	0.5
Nickel	3980 **	10,000	2,400
Zinc	300 **	1,000	100
Copper	22 **	720	130
Lead	5000 *	10,000	620

* Discharge permit levels under RCRA

** Discharge permit levels for DOE-ORO
as specified by region 4 EPA

Incubation 14 h. at 32 degrees

Sulfate-reducing bacteria have also been associated with the degradation of ethanol, methanol, acetone and toluene (Postgate, 1984). The degradation of these organics appear to occur when sulfate levels in the medium is low. The rates of degradation for these organics remains to be determined. Thus experiments would be necessary to determine the degradation rate and whether this bacteria can degrade some of the other organics shown in Table 1.

For the removal of metals from solution, an aerobic bacteria has recently been identified which will reduce ionic metals to the elemental form. This bacteria has been shown to reduce mercury, silver, selenium, copper, lead, gold, platinum, and cadmium to the elemental form (Figure 2). Results from experiments showing the effects of this bacteria on the above metals are shown in Table 4. The above metals in the chloride form (except for selenium which added as Na_2SeO_3) were added to medium containing the bacteria Pseudomonas maltiphila (02) and incubated at 32 °C for 14 h.. After incubation, the samples were filtered through an 0.45 μ filter and the filtrate was analyzed for the above metals. As shown, the soluble concentration of most of these metals were reduced by 10-fold. This bacterium has also been used to treat photographic fixer waste with results showing that silver in the fixer could be reduced to 0.005 ppm (the initial silver concentration was 10,000 ppm). The bacterium has been shown to grow on either methanol and ethanol alone, which suggests that it can degrade these organics.

Microbial Reduction of Metals to the Elemental Form

Reaction:

$++\text{Hg} + \text{BACTERIA} \quad (2 \text{ electrons}) \quad \rightarrow$

$0\text{Hg} + \text{BACTERIA}$

0Hg in aqueous solution is removed by
cross flow filtration (0.45 μ filter)

OTHER METALS

Lead	(2+)
Cadmium	(2+)
Silver	(1+)
Tin	(2+)
Selenium	(2+)
Gold	(1+)
Copper	(2+)

Effective in Aqueous Solution

BACTERIAL REDUCTION OF Pb⁺⁺, Hg⁺⁺, Se⁺ Ag⁺⁺, Pt⁺⁺, and Cu⁺⁺ to ELEMENTAL METAL

METAL	INITIAL ppm	FINAL ppm
Pb ⁺⁺	922	50
Hg ⁺⁺	3000	6.2
Se ⁺⁺	4500	33
Ag ⁺⁺	5800	1.8
Pt ⁺⁺	1100	19
Cu ⁺⁺	3600	80

BACTERIA INCUBATED IN MEDIUM WITH THESE METALS FOR 14 h. AT 32°C. THIS MIXTURE WAS FILTERED AND FILTRATE ANALYZED FOR THESE METALS

Several microbial systems have been investigated that reduce the solubility of inorganics in aqueous waste. These inorganics can be removed from the aqueous waste by ultra filtration. In contrast, only a few microorganisms have been identified that can degrade most of the organics shown in Table 1. Furthermore, results showing that microorganisms can degrade the organics glutaraldehyde, chlorodifluoromethane and trichlorotrifluoroethane remain to be reported. However, through genetic engineering one may develop an organism that can degrade these three organics. Thus it would seem highly plausible that microorganisms could be used to treat the aqueous waste generated in experiments aboard the space station.

Clearly, additional studies would be necessary to design a bioreactor for treating the aqueous waste. However, as discussed above, biological waste treatment systems are effective in reducing the concentration of hazardous waste and may be considerably less expensive than the chemical and physical technologies currently available for waste treatment. In summary, Tables 5, 6 and 7 describe the current technologies that may be applied to waste treatment, and provide examples of how biological systems may be used in treating waste on the space station.

POTENTIAL TREATMENT METHODS FOR WASTE ON THE SPACE STATION

TREATMENT METHODS:

- 1) Filtration
 - a. ultra
 - b. charcoal (organics)
 - c. resins (inorganics)
- 2) Store and return to earth
- 3) Chemical and/or physical methods
- 3) Biological methods

BIOLOGICAL PROCESSES IN MICR.

FOR WASTE TREATMENT

PROCESSES

ADSORPTION (Cell surface)

ABSORPTION (internalize)

CONVERSION (sulfide or elemental)

METABOLISM (carbon dioxide and
water or less toxic
metabolite)

BIOREACTOR FOR THE REMOVAL OF CONTAMINANTS IN SPACE STATION AQUEOUS WASTE

BIOREACTORS

Anaerobic

1. Sulfate-reducing bacteria, aqueous waste (metals + organics), nutrients ----->
2. Solution containing metal sulfides
3. Filtration of this solution -----> metal sulfide sludge + degraded organics + reusable water

Aerobic

1. *Pseudomonas Maltiphila* (O2), aqueous waste (metals + organics), nutrients ----->
 2. Solution containing elemental metals
 3. Filtration of this solution -----> sludge containing elemental metal + degraded organics + reusable water
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1. *Pseudomonas* (Putida B-13), aqueous organic waste, nutrients ----->
 2. Solution containing degraded organic
 3. Filtration of this solution -----> bacterial sludge

REFERENCES

Hirschhorn, J. S. et al. Technologies and Management Strategies for Hazardous Waste Control, OTA-M-196 Office of Technology Assessment Washington D. C. March 1983

Alexander, M. 1981. *Science* 211: 132

Cook, A. M., Grossenbacher, H. and Huetter, R. 1983. *Experientia* 39: 1191

Ahmed, M. and Focht, D. D. 1973. *Can. J. Microbial.* 19: 45

Fedorak, P. and Westlake, D. W. 1984. *Appl. Environ. Microbial.* 47: 858

Johnson, L. M. and Talbot, H. W. 1983. *Experientia* 39: 1236

Revis N. W., Osborne, T. and Holdsworth, G. In Press

Postgate, J. R. 1984. The sulfate-reducing bacteria, 2nd Ed. Cambridge University Press, Cambridge, UK.

Brown, M. P., Rush, B., Rhee, G. Y. and Shane, L. 1988. *Science* 240: 1974

Rajo, F. et al. 1988. *Science* 238: 1395